AD-A164 675 PRIMARY EYE IRRITATION POTENTIAL OF NITROGUANIDINE IN RABBITS(U) LETTERMAN ARMY INST OF RESEARCH PRESIDIO OF SAN FRANCISCO CA G F HIATT ET AL. 09 JAN 86 LAIR-209 1/1

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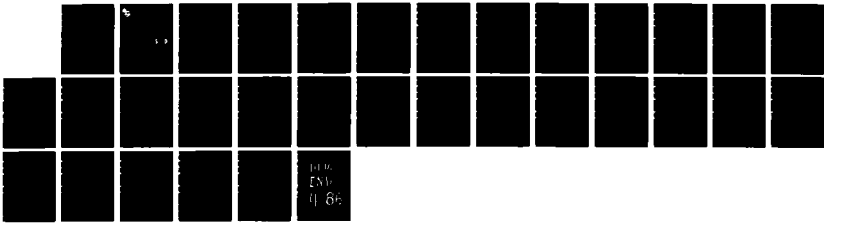

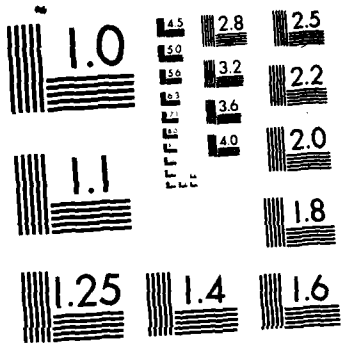

INSTITUTE REPORT NO. 209

PRIMARY EYE IRRITATION POTENTIAL OF NITROGUANIDINE IN RABBITS

GERALD F. S. HIATT, PhD
STEVEN K. SANO, SGT, BA
and
DON W. KORTE JR, PhD, MAJ MSC

TOXICOLOGY GROUP
DIVISION OF RESEARCH SUPPORT

JANUARY 1986

Toxicology Series 118

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Primary eye irritation potential of nitroguanidine in rabbits. Toxicology Series 118. -- Hiatt, Sano, and Korte

In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. *(AR 360-5)*

Edwin S Seaton 9 Jun 86

(Signature and date)

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE	READ INSTRUCTIONS BEFORE COMPLETING FORM
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18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Nitroguanidine, Eye Irritation, Ocular Irritation, Mammalian Toxicology, Rabbits, Ocular Toxicity.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The potential of nitroguanidine to produce primary eye irritation was evaluated in male New Zealand White rabbits by using a modified Draize method. Nitroguanidine produced no response indicative of a potential to cause irritation upon direct contact with the eye. Slight conjunctival vasodilation was the most serious response observed.

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ABSTRACT

The potential for nitroguanidine to produce primary eye irritation was evaluated in male New Zealand White rabbits by using a modified Draize method. Nitroguanidine produced no response indicative of a potential to cause irritation upon direct contact with the eye. Slight conjunctival vasodilation was the most serious response observed.

Key Words: Nitroguanidine, Eye Irritation, Ocular Irritation, Mammalian Toxicology, Rabbits, Ocular Toxicity

PREFACE

TYPE REPORT: Primary Eye Irritation GLP Study Report

TESTING FACILITY: US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR: US Army Medical Research and Development Command
US Army Medical Bioengineering Research
and Development Laboratory
Fort Detrick, Maryland 21701-5010
Project Officer: Jesse Barkley, MS

WORK UNIT: 3E162720A835; WU 180; APC TL09

GLP STUDY NUMBER: 84024

STUDY DIRECTOR: MAJ Don W. Korte Jr, PhD, MSC

PRINCIPAL INVESTIGATOR: Gerald F.S. Hiatt, PhD

CO-PRINCIPAL INVESTIGATOR: Steven K. Sano, SP4, BA

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocol, SOPs, raw data, analytical, stability, and purity data of the test compound, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: Nitroguanidine

INCLUSIVE STUDY DATES: 6 Sept - 5 Oct 1984

OBJECTIVE: The objective of this study was to determine the primary eye irritation potential of nitroguanidine in male New Zealand White rabbits.

DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

15 August 1985

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

1. The report and raw data for GLP Study 84024 were audited on 3 June 1985.

2. I have reviewed the revised manuscript of this study and find that it meets the requirements of the EPA Good Laboratory Practice regulations.

GARY L. DUTCHER
SP6, USA
Quality Assurance Unit

TABLE OF CONTENTS

Table of Contents (continued)

Primary Eye Irritation of Nitroguanidine in Rabbits -- Hiatt et al

Nitroguanidine is being evaluated by the US Army as a replacement for the nitrocellulose component of certain propellants/munitions. The US Army Medical Bioengineering Research and Development Laboratory (USAMBRDL) was assigned the mission of evaluating the "health effects" of nitroguanidine. As part of their mandate, USAMBRDL has tasked the Toxicology Group, LAIR, to develop a toxicologic profile for nitroguanidine, and intermediates/by-products of its manufacturing process, in accordance with the Toxic Substances Control Act regulations promulgated by the Environmental Protection Agency (EPA). This study was conducted to evaluate the primary ocular toxicity of nitroguanidine.

Objective of Study

The objective of this study was to determine the primary eye irritation potential of nitroguanidine in male New Zealand White rabbits.

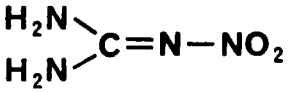
MATERIALS

Test Substance

Chemical name: Nitroguanidine

Chemical Abstracts Service Registry No.: 556-88-7

Molecular structure:



Molecular formula: $CH_4N_4O_2$

Other test substance information is presented in Appendix A.

Vehicle

None

Animal Data

Six male New Zealand White rabbits (Elkhorn Rabbitry, 5265 Starr Way, Watsonville, CA) were identified individually with ear tattoos numbered 84F576, 84F580, 84F581, 84F582, 84F583, and 84F590. Animal weights on dosing day ranged from 2.4 to 3.5 kg. Additional animal data appear in Appendix B.

Husbandry

Rabbits assigned to this study were housed individually in stainless steel, screen-bottomed, battery-type cages with automatically flushing dump tanks. The diet consisted of approximately 150 g/day of Certified Purina Chow Diet 5322 (Ralston Purina Company, Checkerboard Square, St. Louis, MO); water was provided by continuous drip from a central line. Temperature in the animal room was maintained at 14.4 to 17.8°C with a relative humidity range of 40 to 70 percent, except for an 8-hour period on 22 Sep 84 when the temperature fluctuated between 12.8 and 26.7°C. The photoperiod was 12 hours of light per day.

METHODS

Conduct of this study was in accordance with the LAIR Standard Operating Procedure "Primary Eye Irritation Study" (1) and guidelines promulgated by the EPA for ocular irritation testing (2).

Group Assignment/Acclimation

Study rabbits were divided into two dose groups of 3 males each. Following a 14-day quarantine, the animals were acclimated for at least 5 days before dosing. During this period they were observed daily for signs of illness.

Dosage Levels and Administration

One-tenth milliliter (0.025 g) of nitroguanidine was administered once to the treated eye of each rabbit by gently pulling the lower lid away from the conjunctival cul-de-sac to form a cup into which the compound was dropped. Upper and lower lids were then held gently together for one second to prevent loss of material. Group 1 was dosed on 25 Sep 84 and Group 2 was dosed on 2 Oct 84.

Compound Preparation

EPA guidelines express ocular test compound doses in terms of volume (milliliters). Since nitroguanidine was administered in

crystalline powder form, it was necessary to equate milliliters to grams. Using a pipette, it was determined that 0.1 ml of tightly packed nitroguanidine weighed 0.025 g. Therefore, aliquots of nitroguanidine weighing 0.025 g were used for dosing.

Test Procedures

On 24 Sep 84, both eyes of Group 1 animals were examined, for any pre-existing abnormalities, by the procedure detailed below. For each animal, the eye with the nearest normal appearance was designated for treatment; the contralateral eye served as an untreated control. On the next day, 0.025 g nitroguanidine was placed in the treated eye of each rabbit in this group. Group 2 rabbits underwent the same procedures on 1 and 2 Oct 84, respectively.

Ocular Examination/Grading

Initially each eye was observed unaided in a darkened room with focal illumination (pen light). Structures examined included: the lids and surrounding fur, the conjunctiva (semilunar, palpebral and bulbar), the cornea, and the iris. Grading of the cornea, iris and conjunctiva was performed according to Table 1 (modified from Reference 3). During the 24, 48 and 72-hour observations, each eye was also examined with use of a slit lamp. Special attention was given to integrity of the corneal surface, thickness of the corneal stroma, clarity of anterior chamber fluid, iridial morphology, clarity of the lens, and lenticular surface morphology (4). Additionally, any areas appearing grossly abnormal were examined under high magnification. All observations, including normal appearance, were detailed on the grading sheet. Following this, fluorescein dye (Fluor-I-Strips, Ayerst Laboratories Inc., New York, N.Y. 10017) was introduced onto the eye, which was then observed under ultraviolet light. Any corneal areas reacting with the dye (a sign of discontinuity of the corneal epithelium) were described with respect to area and intensity of fluorescence. Examination and grading of ocular reactions were performed in this fashion at 1, 4, 24, 48, and 72 hours after dosing. Fluorescein staining was omitted from the 1 and 4-hour observations, as was slit lamp examination. Due to an almost total lack of reaction during the 72 hours after dosing, the study was terminated, according to protocol, after this observation. Therefore no scoring or observations were performed at 7, 14 or 21 days.

Duration of Study

Appendix C is a complete listing of historical events.

Changes/Deviations

Except for the following deviations, all aspects of this study were performed according to the approved protocol and any relevant amendments and SOPs.

TABLE 1

GRADES FOR OCULAR LESIONS

CORNEA

Opacity: degree of density (area of greatest density taken for reading) No ulceration or opacity.....0

Scattered or diffuse areas of opacity (other than slight dulling of normal luster) details of iris clearly visible.....1*

Easily discernible translucent areas, details of iris slightly obscured.....2

Nacreous areas, no details of iris visible, size of pupil barely discernible.....3

Opaque cornea, iris not discernible through opacity.....4

IRIS

Normal.....0

Markedly deepened rugae, congestion, swelling, moderate circumiridial hyperemia or injection, any of these or any combination thereof, iris still reacting to light (sluggish reaction is positive).....1*

No reaction to light, hemorrhage, gross destruction (any or all of these).....2

CONJUNCTIVA

Redness (refers to palpebral and bulbar conjunctiva, excluding cornea and iris)

Blood vessels normal.....0

Some blood vessels definitely hyperemic (injected).....1

Diffuse, crimson color, individual vessels not easily discernible....2*

Diffuse beefy red.....3

Chemosis: lids and/or nictitating membranes

No swelling.....0

Any swelling above normal (including nictitating membranes).....1

Obvious swelling with partial eversion of lids.....2*

Swelling with lids about half-closed.....3

Swelling with lids more than half-closed.....4

*Indicates minimum level for a positive response

Slit lamp examination (procedure detailed above) was added to the standard observation procedures. Use of the slit lamp enables detection of subtle reactions not grossly observable and better evaluation of those abnormalities which are grossly observable. Color photographic documentation was not performed due to lack of significant response to test compound. With these exceptions, this study was completed in accordance with the appropriate protocol and addenda.

A computer malfunction on 22 Sep 84 resulted in an 8-hour period of spiking in temperature (20.6 to 26.7°C) and humidity (36 to 66%) in the animal room.

Animals were fed approximately 150 grams/day of rabbit chow; access to food ad libitum, as specified in the protocol, resulted in diarrhea in many of the animals.

It is believed that none of these deviations had a negative impact on the performance of the study or the validity of its results.

Raw Data and Final Report Storage

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

RESULTS

Draize-type ocular grading results and slit lamp observations for each rabbit are presented in Appendix D.

Cornea

Nitroguanidine produced no grossly observable effects in the cornea. All treated eyes were assigned zero scores for both opacity and area involvement at all observations after dosing.

Slit lamp examination revealed no corneal reactions referrable to the test compound. One very small punctate lesion at the limits of visability even with slit lamp magnification was observed in one treated eye (84F581) 48 hours after dosing. This same eye, along with its contralateral control, evidenced a fine diffuse stippling (visible only with the slit lamp) over the cornea before dosing. All other slit lamp observations revealed corneas of normal thickness (indicating lack of edema) with smooth surfaces (indicating epithelial integrity). Except for the small lesion, no fluorescein staining was obtained on any of the treated corneas. Staining of this one lesion was readily observable only with the slit lamp.

Iris/Anterior Chamber

No grossly observable reactions were produced in the iris by nitroguanidine. Iridial scores were therefore consistently zero at all observation times.

No iridial abnormalities were detected by slit lamp examination of the treated eyes. Circumiridial vessels and surface morphology were observed to be normal at all times after dosing. Close examination of anterior chamber fluid revealed no evidence of the presence of protein or cells (signs of iridial inflamation).

Lens

The lens is not scored under the Draize-type grading system because of the difficulty in making unaided observations. At all times after dosing, the lens appeared normal during slit lamp examination. No changes were observed in clarity or surface morphology.

Conjunctiva

In this study, nitroguanidine consistently produced only one grossly observable response -- that was slight conjunctival injection. At 1 and 4 hours after dosing, most of the treated eyes exhibited slight vasodilation in the bulbar (sclera) or semilunar (nictitating membrane) conjunctiva. Conjunctival redness scores of 1 were assigned in 5 of 6 treated eyes at these times. At no time during the study was any chemosis (conjunctival swelling) observed.

Slit lamp examination confirmed the presence of dilated vessels within the outer layers of the sclera and the nictitating membrane. No other conjunctival changes were observed.

Control Eyes

At no time during the study did the contralateral, untreated eyes exhibit any change from the pre-dosing examination. Fine diffuse stippling was present before and after dosing in the control eye of one rabbit (84F581). All other untreated controls were normal throughout the entire study.

DISCUSSION

Determination of the potential for ocular damage upon accidental contact of a chemical with the eye is the primary goal of ocular toxicity testing. For this purpose the Draize-type irritation test, used in the present study, is well-suited. An important feature of this test is that the route and type of exposure (ocular instillation followed by a forced blink) closely mimics potential human exposures.

Consumer Product Safety Commission Guidelines, which the EPA recommends for ocular irritation testing, state that an animal is considered to have exhibited a positive reaction if the test substance produces one or more of the following signs: ulceration of the cornea (other than a fine stippling); opacity of the cornea (other than a slight dulling of the normal luster); inflammation of the iris (other than a slight deepening of the rugae or a slight hyperemia of the circumcorneal blood vessels); an obvious swelling in the conjunctiva with partial eversion of the lids; or a diffuse crimson-red coloration in the conjunctiva with individual vessels not easily discernible (1).

Guidelines for classification of chemicals as ocular irritants or non-irritants have been published and form the basis for evaluation in the the present study (5). These Interagency Regulatory Liaison Group (IRLG) guidelines state: "[a] test result is considered positive if four or more animals exhibit a positive reaction. If only one animal exhibits a positive reaction, the test result is regarded as negative." A positive result is defined by the same criteria.

In this study, nitroguanidine produced no positive reaction as defined by the IRLG. Slight conjunctival redness, indicating mild inflammation, was the only response observed. This reaction, although scorable, did not achieve sufficient severity to warrant consideration as a "positive response." A likely explanation for this slight redness was the observed presence in the lower conjunctival cul-de-sac (the site of gradable redness) of small amounts of the test material. Most of the eyes with gradable redness exhibited undissolved nitroguanidine. Therefore physical irritation, not pharmacological activity, may have been responsible for the slight response observed. Since human exposures would be closely followed with thorough washing, even this slight response may not occur.

CONCLUSION

In the present study, nitroguanidine produced no response indicative of a potential to cause ocular irritation upon direct contact with the eye.

RECOMMENDATION

Nitroguanidine should undergo further toxicity testing in accordance with the Toxic Substances Control Act Regulations.

REFERENCES

1. Primary Eye Irritation Study. LAIR Standard Operating Procedure OP-STX-33, Letterman Army Institute of Research, Presidio of San Francisco, CA, 15 June 1984.

2. Environmental Protection Agency. Office of Pesticides and Toxic Substances, Office of Toxic Substances (TS-792). Primary eye irritation. In: Health effects test guidelines. Washington, DC: Environmental Protection Agency, August 1982; EPA 560/6-82-001.

3. Draize JH, Woodard G, Calvery HO. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther 1944;82:377- 390.

4. MacDonald TO, Baldwin HA, Beasley CH. Slit lamp examination of experimental animal eyes. I. Techniques of illumination and the normal animal eye. J Soc Cosmet Chem 1973;24:163-180.

5. Interagency Regulatory Liaison Group. Recommended guidelines for acute eye irritation testing. January 1985.

Page

CHEMICAL DATA

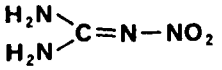
Chemical name: Nitroguanidine (NGu)

Other listed names: Guanidine, Nitro; alpha-Nitroguanidine; beta-Nitroguanidine

Chemical Abstracts Service Registry No.: 556-88-7

LAIR Code: TP036

Structural formula:



Molecular formula: $CH_4N_4O_2$

Molecular weight: 104.1

pH of a saturated aqueous solution: 5.7

Physical state: White Powder

Melting point: 232°C*

Name of contaminants and percentages: Data sheet attached.

Source: Hercules Aerospace Division
Sunflower Ammunition Plant
DeSoto, Kansas

Lot No.: SOW83H001-004

Analytical data/purity: An infra-red spectrum was obtained upon receipt of the compound and major absorption peaks were observed at 3330 (broad), 1660, 1630, 1525, 1400, 1300, 1050, and 780 cm^{-1}.† The spectrum was identical to the Sadtler spectrum for nitroguanidine.‡

* Fedoroff BT, Sheffield OE. Encyclopedia of explosives and related items. Vol 6. Dover, New Jersey: Picatinny Arsenal, 1975: G154.

† Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p. 39. Letterman Army Institute of Research, Presidio of San Francisco, CA.

‡ Sadtler Research Laboratory, Inc. Sadtler standard spectra. Philadelphia: The Sadtler Research Laboratory, Inc., 1962: Infra-red spectrogram #21421.

Stability: An aqueous solution of NGu (48.1 umolar) was prepared and the absorption at 264 nm determined to be 0.689. Three weeks later the same solution was re-examined spectroscopically and the absorption at 264 nm found to be 0.689. A full spectrum scan revealed the characteristics pattern of absorption in the UV range with peak maxima at 215 and 264 nm. These data indicate that NGu is stable in aqueous solution for at least three weeks.*

* Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010, pp. 22 and 36. Letterman Army Institute of Research, Presidio of San Francisco, CA.

DESCRIPTION SHEET FOR EXPLOSIVES, CHEMICALS, ETC

(DRSAR-P-702-109)

DLR CONTROL SYMBOL: EXEMPT-Para 7-2a AR 335-15 | PAGE: 1 OF 1

TO: Commander, US Army Armament Munitions and Chemical Command, Attn: DRSMC-QAD, Rock Island, ILL. 61201

FROM: Sunflower Army Ammunition Plant, DeSoto, Kansas 66018

DATE: September 13, 1983

MATERIAL: Nitroguanidine Type II, Class 2 *

MANUFACTURER: Hercules Aerospace Division, Hercules Incorporated

CONTRACT NO.: DAAA-09-77-C-4016, CLIN 0270

SECTION A - DESCRIPTION OF LOTS

FROM NUMBER	THRU NUMBER	TOTAL NO. LOTS	TOTAL NET AMOUNT ACCEPTED
SOW83H001-004		1	7,000 lbs.

PLACE MANUFACTURED: Sunflower Army Ammunition Plant, DP Facility

SPECIFICATION AND AMENDMENT/DRAWING NO.: MIL-N-494A w/Int. Amend 6 (AR) dated 25 March 1981 *

SECTION B - DESCRIPTION OF MATERIAL

Property	Requirement Min.	Requirement Max.	Analysis
Purity, %	99.0		99.6
Ash Content, %		0.30	0.03
pH Value	4.5	7.0	7.55 **
Acidity (as H_2SO_4), %		0.06	ND ***
Total Volatiles, %		0.25	0.03
Sulfates (as $NaSO_4$), %		0.20	0.01
Impurities, H_2O Insoluble, %		0.20	0.01
Particle Size, Microns		3.0 *	4.0 ****
Particle Size, Std. Dev.		± 0.5	0.168

* As amended by Contract Scope of Work
** Approved by Waiver No. NQ83-1 dated Sept. 2, 1983
*** ND = None Detected
**** Approved by Waiver No. NQ83-2 dated Sept. 9, 1983

REMARKS

1.) Manufactured under SOW ES 1A-3-8423, Nitroguanidine Particle Size, dated 1 Feb. 83.

2.) Packaging: Level B - fiber drums to Spec. DOT 21C60. Drums numbered 3 thru 243 and 247 thru 285. 25 pounds per drum per HAD letter dated Aguust 1, 1983, to COR.

SECTION C - CERTIFICATION

SAMPLING CONDUCTED BY: Hercules Aerospace Division

TESTING CONDUCTED BY: Hercules Aerospace Division

THE ABOVE MATERIAL COMPLIES WITH ALL SPECIFICATION REQUIREMENTS AND IS CERTIFIED TRUE AND CORRECT.

13 Sept 83 (DATE) A. W. English (SIGNATURE)

THE ABOVE DESCRIBED LOTS ARE HEREBY ACCEPTED

FOR THE COMMANDER

14 Sep 83 (DATE) Quality Assurance Specialist (TITLE) M. A. Kozan (SIGNATURE)

ARRCOM FORM 213-R, 10 AUG 77 — REPLACES ARMCOM FORM 213-R, 15 JUL 75 (TEMP) WHICH MAY BE USED.

APPENDIX A (concluded)

ANIMAL DATA

Species: *Oryctolagus cuniculus*

Strain: New Zealand White (albino)

Source: Elkhorn Rabbitry
5265 Starr Way
Watsonville, CA 95076

Sex: Male

Age: Young Adults

Animals in each group: 2 groups of 3 males each

Condition of animals at start of study: Normal

Body weight range at dosing: 2.4 - 3.5 kg

Identification procedures: Ear tattoo numbers 84F576, 84F580, 84F581, 84F582, 84F583, 84F590.

Pretest conditioning:

1. Quarantine from 6 Sept - 19 Sept 1984

2. Animal eyes were examined 24 hours before dosing using slit lamp, fluorescein dye and ultraviolet light.

Justification: Laboratory rabbits are a proven sensitive animal model for ocular testing.

HISTORICAL LISTING OF STUDY EVENTS

Date	Event
6 Sep 84	Animals arrived at LAIR. They were tattooed, weighed, examined for illness, placed under a two week quarantine, given one application of Canex®/mineral oil into ears for earmite prevention. No animals died during the quarantine period.
6-19 Sep 84	Animals checked daily by Animal Resources Group (ARG) personnel.
19 Sep 84	Rabbits certified healthy by ARG Staff Veterinarian and removed from quarantine, separated into test groups and weighed.
24 Sep 84	Animals checked for pre-existing ocular injury (Group 1).
25 Sep 84	Group 1 rabbits were dosed according to test chemical group and weighed. One and four hour post-exposure scores performed.
26 Sep 84	24-hour post-exposure scores performed (Group 1).
27 Sep 84	48-hour post-exposure scores performed (Group 1).
28 Sep 84	72-hour post-exposure scores performed. Study was terminated and animals weighed and sacrificed (Group 1).
1 Oct 84	Animals checked for pre-existing ocular injury (Group 2).
2 Oct 84	Group 2 Rabbits were dosed according to test chemical group and weighed. One and 4-hour post-exposure scores performed.
3 Oct 84	24-hour post-exposure scores performed (Group 2).
4 Oct 84	48-hour post-exposure scores were performed (Group 2).
5 Oct 84	72-hour post-exposure scores performed. Study was terminated, animals were weighed and sacrificed (Group 2).

TABULAR SCORING DATA

ON

ACUTE EYE IRRITATION SUMMARY FORMS

FIGURE 1

SCORING SUMMARY - ACUTE OCULAR IRRITATION

TISSUE: CORNEA (OPACITY)

GLP STUDY: 84024 P.I.: GERALD F.S. HIATT

COMPOUND: NITROGUANIDINE

PHYSICAL STATE: POWDER QUANTITY: 0.025 GM

DOSING DATE: 25 SEP, 2 OCT 1984

SPECIES: Rabbit

SCORE BY ANIMAL

Rabbit #	Pre	1 h	4 h	24 h	48 h	72 h	7 d	14 d	21 d
84F581	0	0	0	0	0	0	NA	NA	NA
84F583	0	0	0	0	0	0	NA	NA	NA
84F530	0	0	0	0	0	0	NA	NA	NA
84F576	0	0	0	0	0	0	NA	NA	NA
84F580	0	0	0	0	0	0	NA	NA	NA
84F582	0	0	0	0	0	0	NA	NA	NA

FIGURE 2

SCORING SUMMARY - ACUTE OCULAR IRRITATION

TISSUE: CORNEA (AREA INVOLVEMENT)

GLP STUDY: 84024 P.I.: GERALD F.S. HIATT

COMPOUND: NITROGUANIDINE

PHYSICAL STATE: POWDER QUANTITY: 0.025 GM

DOSING DATE: 25 SEP, 2 OCT 1984

SPECIES: Rabbit

SCORE BY ANIMAL

Rabbit #	Pre	1 h	4 h	24 h	48 h	72 h	7 d	14 d	21 d
84F581	0	0	0	0	0	0	NA	NA	NA
84F583	0	0	0	0	0	0	NA	NA	NA
84F590	0	0	0	0	0	0	NA	NA	NA
84F576	0	0	0	0	0	0	NA	NA	NA
84F580	0	0	0	0	0	0	NA	NA	NA
84F582	0	0	0	0	0	0	NA	NA	NA

FIGURE 3

SCORING SUMMARY - ACUTE OCULAR IRRITATION

TISSUE: IRIS

GLP STUDY: 84024 P.I.: GERALD F.S. HIATT

COMPOUND: NITROGUANIDINE

PHYSICAL STATE: POWDER QUANTITY: 0.025 GM

DOSING DATE: 25 SEP, 2 OCT 1984

SPECIES: Rabbit

SCORE BY ANIMAL

Rabbit #	Pre	1 h	4 h	24 h	48 h	72 h	7 d	14 d	21 d
84F581	0	0	0	0	0	0	NA	NA	NA
84F583	0	0	0	0	0	0	NA	NA	NA
84F590	0	0	0	0	0	0	NA	NA	NA
84F576	0	0	0	0	0	0	NA	NA	NA
84F580	0	0	0	0	0	0	NA	NA	NA
84F582	0	0	0	0	0	0	NA	NA	NA

FIGURE 4

SCORING SUMMARY - ACUTE OCULAR IRRITATION

TISSUE: CONJUNCTIVA (REDNESS)

GLP STUDY: 84024 P.I.: GERALD F.S. HIATT

COMPOUND: NITROGUANIDINE

PHYSICAL STATE: POWDER QUANTITY: 0.025 GM

DOSING DATE: 25 SEP, 2 OCT 1984

SPECIES: Rabbit

SCORE BY ANIMAL

Rabbit #	Pre	1 h	4 h	24 h	48 h	72 h	7 d	14 d	21 d
84F581	0	1	1	0	0	0	NA	NA	NA
84F583	0	1	1	0	0	0	NA	NA	NA
84F590	0	1	1	0	0	0	NA	NA	NA
84F576	0	1	1	0	0	0	NA	NA	NA
84F580	0	1	1	0	0	0	NA	NA	NA
84F582	0	0	0	0	0	0	NA	NA	NA

FIGURE 5

SCORING SUMMARY - ACUTE OCULAR IRRITATION

TISSUE: CONJUNCTIVA (CHEMOSIS)

GLP STUDY: 84024 P.I.: GERALD F.S. HIATT

COMPOUND: NITROGUANIDINE

PHYSICAL STATE: POWDER QUANTITY: 0.025 GM

DOSING DATE: 25 SEP, 2 OCT 1984

SPECIES: Rabbit

SCORE BY ANIMAL

Rabbit #	Pre	1 h	4 h	24 h	48 h	72 h	7 d	14 d	21 d
84F581	0	0	0	0	0	0	NA	NA	NA
84F583	0	0	0	0	0	0	NA	NA	NA
84F590	0	0	0	0	0	0	NA	NA	NA
84F576	0	0	0	0	0	0	NA	NA	NA
84F580	0	0	0	0	0	0	NA	NA	NA
84F582	0	0	0	0	0	0	NA	NA	NA

Figure 6

Clinical Description of Eye Lesions by Animal and Time

GLP Study No. 84024 — Chemical Name Nitroguanidine

Principal Investigator Gerald F.S. Hiatt — Physical State Crystalline Powder

Date Started 6 Sept 84 — Amount Applied 0.1 ml 0.025 gm

Animal 84F___ ___ ___

Clinical Sign	Pre	1 hr	4 hr	24 hr	48 hr	72 hr	7 d	14 d	21 d
Very fine, diffuse Corneal stipling	581	581	581	581	581	581	NA	NA	NA
Test Compound present in conjunctival sac	-	576 580 581 582 583 590	580 581 582 583	583	-	-	NA	NA	NA
Slight Scleral or Semilunar Vasodilatation	-	576 580 581 583 590	576 580 581 583 590	-	-	-	NA	NA	NA
Microscopic Corneal Punctate Lesion	-	-	-	-	581	-	NA	NA	NA

SUMMARY OF OCULAR OBSERVATIONS

One Hour Post-Dosing:

Slight hyperemia was present in 5 of the 6 test rabbits. This hyperemia was confined to the lower conjunctiva, both bulbar and palpebral, and was visible with the unaided eye. Small to large masses of the test compound were observed in the lower conjunctival sac in the same 5 eyes at this time.

All other structures appeared normal.

Four Hours Post-Dosing:

Slight hyperemia continued to be present in the lower conjunctiva of the same 5 rabbits. In one eye, the severity of redness had decreased somewhat. Test compound remained in the lower conjunctival sac of only 2 rabbits.

All other structures appeared normal.

Twenty-four Hours Post-Dosing:

By this time, the hyperemic reaction had completely disappeared in the affected eyes. Eyelids, nictitating membrane, conjunctiva, cornea, anterior chamber, iris and lens of each treated eye appeared normal, even by slit lamp examination. All eyes were judged normal overall.

No fluorescein staining was present.

Forty-eight Hours Post-Dosing:

All structures examined by slit lamp appeared normal and no fluorescein staining was present.

Seventy-two Hours Post-Dosing:

All structures examined by slit lamp appeared normal and no fluorescein staining was present.

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